REMARKS

1. Claim amendments

Claim 1 has been limited to include the features of claims 2, 3, 4, 6, 7 and 8. Accordingly, the amendment of claim 1 raises no new issues of patentability. The limitation of claim 1 and the cancellation of claims 2-10 are made without prejudice or disclaimer.

Claims 17 and 18 have been added, as discussed in section 2. Thus, there is a net decrease of seven claims.

2. Restriction Requirement

The Examiner has required that non-elected claims be cancelled in this response or other suitable action taken.

The requirement is respectfully traversed as premature. The claims of elected group I are product claims, and we continue to maintain that claim 1 is allowable. The claims of withdrawn group II (claims 14-16) are method claims dependent, directly or indirectly, on claim 1. Hence, if claim 1 is deemed allowable, claims 14-16 will be rejoined pursuant to MPEP 821.04.

All group I claims are generic to, or otherwise read upon, the elected species (yeast = Saccharomyces cerevisiae). If a generic claim is deemed allowable, the other species will be rejoined. Applicants continue to argue the allowability of generic claims. Hence, it is premature to amend the claims to limit them to the elected species. However, we have added new claim 17, which is directed to the genus Saccharomyces, and claim 18, to the elected species. This doesn't raise new issues as the search was necessarily directed to the elected species and the Markush group of claim 12 specifically enumerated the genus Saccharomyces and the Markush group of claim 12 specifically enumerated the species S. cerevisiae.

3. Definiteness Issues (OA pp. 7-10)

3.1. The Examiner has argued that the lack of a definition of the term 'native level' in respect of the third enzyme renders

the term unclear. The present amendments avoid that terminology, so the rejection is moot. However, we deny that in the last response the Applicant was arguing limitations not present in the claim at that time and we maintain that the rejection was not justified as the wording questioned was clear on its face.

3.2. Secondly, it has been urged that it is unclear whether the first metabolite is simultaneously transformed into a second metabolite and a third metabolite or whether these are separate metabolic conversions.

Claim 1 requires that there is a first metabolic pathway transforming a first metabolite (now named as glyceraldehyde-3-phosphate) into initially a second metabolite and then into a further metabolite (now named as 3-phosphoglycerate). Further, the claim requires that there is a second metabolic pathway in which glyceraldehyde-3-phosphate is transformed into 3-phosphoglycerate.

So in both pathways, the transformation is:

Glyceraldhehyde-3-phosphate → 3-phosphoglycerate.

We submit that it is plain that any given molecule of glyceraldehyde-3-phosphate can participate only in one or the other pathway.

The two pathways cannot be sequential in that once a molecule of glyceraldehyde-3-phosphate has reacted in the first pathway, it is no longer available to undergo a sequential transformation in the second pathway.

Equally, for any given molecule of glyceraldehyde-3-phosphate, the pathways cannot be simultaneous. The molecule can only react once, so it must participate in either but not both of the two pathways. It cannot be in both pathways at once.

If one considers two different molecules of glyceraldehyde-3-phosphate, it is apparent that it is open for one to react in the first pathway either before, simultaneously with, or after the other molecule reacts in the second pathway.

Taking the whole population of glyceraldehyde-3-phosphate molecules, it is apparent that some will be reacting in the first pathway before, after and simultaneously with others reacting in the second pathway, since both pathways are open to them.

The claim is thus entirely clear.

3.3. The Examiner questioned the parenthesized EC numbers associated with the terms GAPN (EC 1.2.1.9) and GAPDH (EC 1.2.1.12). Since these expressions are no longer present in the claims, we submit that the rejection is moot. However, we also submit that the rejection was erroneous. In justification of the rejection, the Examiner has stated:

'The specification as filed does not disclose the meaning of "GAPN (EC 1.2.1.9" and "GAPDH (EC 1.2.1.12)". However, at page 15, lines 10-15, the specification teaches the overall conversion of glucose to the different metabolites for the GAPN strain. Hence it is unclear whether the EC number generically identifies all the GAPN strains or is strain specific, e.g., Streptococcus, S. cerevisiae and others.'

First, we note that page 15, lines 10-15 does not contain teaching matching the Examiner's description. Page 15 contains a description of Example 2 relating to shake flask cultivation conditions and the genes in question are not mentioned.

Secondly, the specification does not suggest that the EC numbers mean anything other than what they normally mean in this art. In particular, the specification does not suggest that the EC numbers refer to 'a strain'. We agree that on occasions the specification refers to a/the 'GAPN strain', but that is merely shorthand for some particular strain under discussion into which the gapN gene has been engineered. Such references would not appear to be relevant to the clarity of the EC numbers.

4. Obviousness Issues (pp. 10-16)

The Examiner rejects the claimed subject matter (Claims 1-13) as allegedly obvious over Nissen et al in view of Valverde

et al.

Nissen et al is cited as disclosing a metabolically engineered S. cerevisiae wherein reduced formation of surplus NADH and an increased consumption of ATP in biosynthesis results in decreased glycerol yield. To this end, a mutant S. cerevisiae was produced in which GLN1 encoding glutamate synthetase, and GLT1 encoding glutamate synthetase were over expressed. GDH1 encoding the NADPH-dependent glutamate dehydrogenase was deleted.

This led to consumption of 1 mol of NADH and ATP per mole of glutamate instead of 1 mol of NADPH, leading to a reduction of surplus formation of NADH, increased ethanol production and decreased glycerol production.

Nissen et al does not disclose reducing formation of NADH and ATP by the enzymatic activity of a non-phosphorylating dehydrogenase (e.g. GAPN aka GAPDHN). Indeed, as we have previously pointed out Nissen et al does not disclose reducing formation of NADH at all. When Nissen et al speaks of reduced formation of surplus NADH, this is not in fact via the mechanism of reduction of NADH formation, but via provision of a pathway for consuming NADH.

The Examiner's contention was that because Nissen et al taught that reducing formation of NADH and increasing consumption of ATP resulted in decreased glycerol formation in yeast, it would have been obvious from Valverde et al that this effect could be obtained also by expressing GAPN in yeast to produce 3-phosphoglycerate with production of NADPH rather than NADH + ATP (Office Action of 12th March 2008 at page 12, line 4).

As we have previously submitted, Nissen et al's teaching is significantly mis-stated by the Examiner. It does not teach reduction in the production of NADH. Rather, it teaches provision of an NADH consuming reaction path that does not lead to glycerol. This is a different and distinct mechanism for reducing surplus NADH undermines the Examiner's argument, which starts from a false premise.

The Examiner has responded that the argument does not start

from a false premise and has put forward a restatement of Nissen et al's teaching (current office action page 10-11, bridging paragraph). We note, however, that nowhere in that analysis does the Examiner refer to Nissen et al teaching a reduction in the formation of NADH.

The situation in respect of NADH formation and surplus NADH formation upstream of glycerol production may be summarized as follows:

(rate of NADH formation) - (rate of NADH consumption) = (surplus NADH formation)

then

Surplus NADH formation → glycerol production

Nissen et al we agree teaches reduction of the amount of surplus NADH formation. However, it can be seen from the above that this can be achieved in two ways, one can decrease the NADH formation, or one can increase the NADH consumption. The Examiner's statement of the objection indicated that Nissen et al taught to decrease NADH formation, which is we submit untrue. Rather, Nissen et al taught to increase NADH consumption.

The Examiner's supposed rebuttal of this point, bridging pages 10 and 11 of the current office action, discusses only the reduction in Nissen et al of surplus NADH. We did not dispute that Nissen et al teaches reduction of surplus NADH. The issue relates to the nature of the mechanism taught by Nissen et al, which we reiterate is not reduction in NADH production.

The difference between Nissen et al and the claimed invention may be represented as follows:

Nissen et al.	
Before	After
NADH producing reactions	unaffected
NADPH producing reactions	unaffected
NADH consuming reactions	Boosted: GLN1 and GLT1 over expressed, boosting ammonium + 2-oxoglutarate + NADH = glutamate + NAD
NADPH consuming reactions	Reduced: GDH1 (Glutamate dehydrogenase) deleted, cutting ammonium + 2-oxoglutarate + NADPH = glutamate + NADP
Surplus NADH consuming glycerol production	Reduced
Invention	
NADH producing reactions	Reduced: glyceraldehyde-3-phosphate diverted from glyceraldehyde-3-phosphate + NAD = glyceraldehyde-3-phosphate +NADH
NADPH producing reactions	Boosted: GAPN introduced, boosting glyceraldehyde-3-phosphate + NADP = glyceraldehyde-3-phosphate +NADPH
NADH consuming reactions	Unaffected
NADPH consuming reactions	Unaffected
Surplus NADH consuming glycerol production	Reduced

Secondly, as explained in Nissen et al, one previous strategy for avoiding production of glycerol in yeast was to block the pathway leading to its production by deletion of genes

encoding GPD1 and GPD2, but this led to a strain that could not grow under anaerobic conditions (col. 1, page 70). Nissen et al instead choose to drain off surplus formation of NADH by changing the cofactor requirement in amino acid synthesis.

Clearly, Nissen et al teaches it to be a problem with that earlier strategy that it resulted in a failure to grow under anaerobic conditions. That will have an effect on what a reader of Nissen et al would consider would be an acceptable modification of Nissen et al. The skilled artisan reading Nissen et al would be discouraged by Nissen et al from adopting any steps that would result in failure to grow under anaerobic conditions.

Valverde et al discloses a metabolically engineered E. coli in which the NAD-dependent glycolytic phosphorylating G3P dehydrogenase GAPDH was deleted and in which GAPN was expressed resulting in the reaction:

Glyceraldehyde 3-phosphate + NADP = 3-phosphoglycerate + NADPH.

As a result, the E. coli strain was unable to grow anaerobically on sugars but had recovered the ability to grow aerobically on sugars. It also failed to grow on gluconeogenic substrates (acetate + succinate) and showed a lower growth rate than wild type (col. 1, page 155).

One consequence of the metabolic engineering in Valverde et al is that the resulting E. coli is unable to grow anaerobically, the very problem that Valverde et al were trying to avoid. A skilled artisan would therefore not perceive Valverde et al as offering a teaching likely to be useful in yeast as an alternative strategy for obtaining the objects of Nissen et al (reduced glycerol and increased ethanol).

The Examiner has commented that the claims do not recite any limitation related to anaerobic or aerobic conditions. We respectfully point out that the steps which the skilled artisan is motivated to take based on a reading of the prior art cannot depend on the terms of the Applicant's claims. The skilled

artisan would not change from the increased NADH consumption taught in Nissen et al to the decreased formation of NADH taught in Valverde et al, in part because it would be a natural expectation based on the teaching of Valverde et al that the resulting yeast would not grow under the conditions taught by Nissen et al to be desired. The skilled artisan would see that Nissen et al have described and rejected as inadequate the Björkqvist et al teaching describe in the first column on page 70 because it led to inability to grow under anaerobic conditions.

The Examiner has made comments (page 12, middle paragraph) about the ability for fermentation to take place under aerobic conditions given critical control of oxygen levels. It is unclear where this teaching comes from. It may be that the Examiner is relying on teaching not in the cited documents. However, it is unclear in what way this could add to the motivation of a skilled artisan to adopt a modification of Nissen et al that would be seen as being likely to lead to a limitation to aerobic fermentation.

We commented previously that the Examiner's rejection is dependent on the skilled worker choosing not to adopt the whole of the changes taught by Valverde et al and to apply them to yeast. Thus, the claims require that the yeast should have an intact GAPDH which produces NADH, whereas in Valverde et al, the E. coli had its NAD-dependent GAPDH deleted. We submitted that if it were obvious to combine the teachings of Nissen et al and Valverde et al at all (which we deny), the result would be a yeast in which, per Valverde et al, GAPDH would be deleted and GAPN would be introduced. That however would not meet the requirements of claim 1.

In answer to this, the Examiner has said:

'While Valverde discloses transformation of E. coli gapC mutant, e.g., W3CG, transformed with a functional plant GAPDHN, Valverde additionally teaches transformation of E.coli with the GapN gene (i.e., irreversibly oxidises G3P to 3-PGA) and

consequently high production of plant GAPDHN or GAPN …in the presence of the IPTG inducer (p. 153, col. 2, last paragraph; p. 155, col. 1, last paragraph). Note that E.coli comprises endogenous GAPH (GAPDH). Additionally, Valverde teaches E.coli W3CG strains harbouring three gap-2 genes from other bacteria are disclosed at page 156, col. 1, second paragraph. Absent evidence to the contrary, the E. coli transformant comprises both the phosphorylating and non-phosphorylating branches of glycolysis (p. 157, Fig. 4)'

We submit again that Valverde et al does not disclose a strain of E. coli engineered to contain GapN without disablement of production of GAPDH. Page 155, column 1 describing the Valverde et al mutations states 'To avoid any contamination with the host GAPDH we used E. coli gap mutant W3CG, which has the gapC gene disrupted by insertional mutagenesis.

Page 153, column 2, last full paragraph states: 'Functional complementation of the Escherichia coli gap mutant W3CG, which has a Tn10 transposon inserted in the Gap-C like gene gap-2 and lacks GAPDH, has been used.'

Thus, we agree with the Examiner that normally E. coli comprises endogenous GAPDH, but in the work of Valverde et al, this is unambiguously taught to be disabled.

The Examiner relies on page 156, column 1, second paragraph for teaching of mutation of E. coli to harbor three gap-2 genes from other bacteria. These are taught as expressing GAPDH in the mutant E. coli W3CG, which lacks its own GAPDH. It may be that the Examiner is reading this as a disclosure of the insertion into W3CG of both GAPN and GAPDH expression. If so, we submit that this is clearly a mis-reading of Valverde et al. What is disclosed is separate expression in different W3CG mutants of either GAPN or one of the three GAPDH's in question, but not both GAPDH and GAPN from the same mutant.

This is unambiguously clear from Figure 2 B where the expression of the different enzymes is demonstrated by the use

of specific antibodies as described at page 156, column 1, final paragraph.

The results show that in W3CG/pFVNP1 only GAPDHN/GAPN is detected. No GAPDH is seen. In the other three tested W3CG mutants, only GAPDH is seen and not any GAPN. As is said on page 156: 'The presence of only one G3P dehydrogenase protein in the different W3CG-transformed clones was clearly demonstrated.'

The Examiner referred also to Figure 4 as showing both of the two possible branches of glycolysis. However, it can be seen that in Figure 4, the step in the phosphorylating branch mediated by GAPDH (the product of the GapC gene is shown with dashed arrows and the legend explains that this is the reaction in which the W3CG strain is defective.

Accordingly, far from it being the case as alleged by the Examiner that there is a lack of evidence that the Valverde et al mutants lack the simultaneous presence of both of the phosphorylating and non-phosphorylating glycolysis branches, this is completely and unambiguously explicit in Valverde et al.

It is therefore the case that making the combination suggested by the Examiner does not produce anything falling with the Applicant's claim, because a natural yeast engineered per Valverde et al to remove GAPDH capacity and to insert GAPN activity would not meet the claim requirement for native GAPDH, i.e. it would lack the first metabolic pathway of the claim.

Furthermore, we pointed out that it should be recognized that Valverde et al has nothing to say regarding the balance of production of any product by the metabolism of the engineered E. coli, let alone of glycerol/ethanol.

It follows from this that a skilled artisan seeking an alternative technique to that of Nissen et al for improving ethanol production by decreasing glycerol production in yeast would find nothing about that in Valverde et al. He would not therefore be led to consider a combination of these documents. The rejection is essentially based on hindsight and speculation.

The two teachings lie in very different fields, one being

concerned with the metabolism of a yeast and the other with the metabolism of a bacteria. A skilled person would be unlikely to look for an alternative solution to the problem addressed in Nissen et al relating to yeast, in a teaching confined to the metabolism of E. coli.

The Examiner contends that Valverde et al presents the skilled artisan with an alternative method of reducing surplus NADH, which the skilled artisan would readily transfer to adapt the teaching of Nissen et al. However, that Valverde et al provides a method for reducing surplus NADH is purely a conclusion drawn by the Examiner and is not something taught by Valverde et al.

Valverde et al contains no mention of 'surplus NADH' and neither does in discuss any problem or issue connected to the production of surplus NADH or any desire to reduce NADH production. A skilled artisan wishing to find an alternative way of tackling 'surplus NADH' as an issue, whether in yeast or in any other organism, would have no reason to pay attention to Valverde et al. Valverde et al is merely an academic study investigating what glycolytic pathways may be operative in photosynthetic eukaryotes by investigating one particular enzyme's activity in E. coli as a test system. Thus, Valverde et al conclude on page 158 'This metabolic engineering approach has demonstrated the in vivo operation of a non-phosphorylating bypass involving a glycolytic route with no net energy yield that may be functional in photosynthetic eukaryotes and some bacteria'.

Thus, the Examiner's conclusion on page 15 that 'by transforming a yeast with a plasmid encoding GAPDHN (GAPN), wherein the gapC gene encoding GAPDH is mutated, it should be reasonably expected to convert G3P into 3-GPA by yielding one NADPH and not one NADH and one ATP molecules' goes well beyond what is actually taught in Valverde et al and draws conclusions from the teaching of Valverde et al which are shaped by crafting a link between Nissen et al and the invention as claimed in an

exercise of hindsight.

As previously pointed out, Valverde et al does not teach an alternative route by which one can obtain the same effect taught in Nissen et al. Whilst Nissen et al teaches a way to 'drain off' surplus production of NADH that will otherwise lead to glycerol production, no such draining off mechanism is taught or provided in Valverde et al. Instead, Valverde et al teaches the introduction of an NADPH producing pathway. Accordingly, the proposed combination is not a substitution of like with like.

Thus, Valverde et al teaches that the ability to metabolize sugar lost via a deletion of GAPDH can be partially rescued (but only under aerobic conditions) by introducing GAPN. According to the Examiner, it is apparent that the catabolic yield of GAPN includes NADPH. This is clearly quite different from draining off surplus NADH by introducing new NADH consuming reactions as in Nissen et al.

We previously argued also that even if the combination proposed by the Examiner were to be conceived by a skilled reader on the basis of combining Nissen et al and a part of the teaching of Valverde et al as the Examiner proposes, there would have been several reasons not to hold a reasonable expectation that the desired effect would be achieved.

First, it would have been unknown whether GAPN could be expressed successfully and effectively in yeast.

Secondly, it would have been unknown whether production of NADPH via expression and activity of GAPN would have any substantial effect on the level of NADH in yeast. Here it should again be borne in mind that the expression of GAPN does not produce operation of the mechanism taught for reducing glycerol yield in Nissen et al. It does not operate to drain off NADH. Rather the hope on which the Examiner's argument depends would be that avoiding one route to the production of NADH by using GAPN to produce NADPH instead would have a material effect.

This would of course have been completely unknown. First, it would have been unknown to what extent GAPN if expressed

successfully in yeast would become engaged in glycolysis when competing with native yeast enzymes. It should be borne in mind that Valverde et al had deleted the E. coli GAPDH so had not even demonstrated that GAPN would have a material effect in E. coli in which the native glycolysis pathway had not been destroyed, let alone that it would be effective in yeast.

Lastly, as the Examiner has pointed out (page 12 of the action), a second consequence of metabolizing G3P to 3-PGA via GAPN rather than via GAPDH is that one does not get production of ATP. However, the effect on the production of glycerol and ethanol in a yeast of this loss of ATP production would have been quite unknown. As seen in the diagram on page 157 of Valverde et al, ATP is required for consumption in the earlier stages of metabolism of glucose. Valverde et al had reported that their engineered E. coli had a decreased growth rate compared to wild type. A skilled reader would have good grounds for expecting that the hypothesized transformed yeast would also have decreased growth rates.

In answer to these points, the Examiner has first of all stated that 'there is no reason why a functional pea plant GAPDHN (GAPN) couldn't be expressed in transformed yeast with a recombinant DNA'

The Examiner prefaced that conclusion with the words 'As set forth in the paragraph above,' but the paragraph above is a summary of our reasons why it was uncertain whether such a functional pea plant GAPN could be expressed in yeast or have the desired effects. Thus, in reality the Examiner has put forward no counter argumentation.

The Examiner goes on to make a succession of statements which do not seem to be directly relevant to the several points made by the Applicant, which are concerned with the lack of foreknowledge as to whether transferring the GAPN expression to yeast would have the desired results. This is an inherently unpredictable art in which ideas which one might optimistically hope would work can fail to do so for unforeseen reasons. Simply

to assert that there is no reason why a plant gene should not operate as desired in yeast is not enough to dismiss the fact that it could not be known that it would so operate. Nor does it deal with the substantive reasons given by the Applicant for doubting that the proposal would work based on a reading of these documents.

The Examiner states that 'Valverde demonstrated that avoiding one route of production of NADH by expressing a plant GAPN, functionality of the W3CG mutant, which lacks GAPDH is recovered, albeit with different growth and substrate conversion'. Two points arise, first Valverde et al does not make any reference to 'avoiding one route of production of NADH'. It is the Examiner's deduction that this is an effect of what Valverde et al describes. When considering what the reader of Nissen et al would get from Valverde et al, this is an important distinction. Secondly, the Examiner concedes here that the modified W3CG of Valverde et al lacks GAPDH, contrary to the requirements of claim 1. At no point has the Examiner explained why the reader of Nissen et al, choosing to substitute the mutations of Nissen et al with those of Valverde et al should deviate from Valverde et al by omitting the GAPDH deletion of Valverde et al or by reversing it. Yet without this, the result of the combination does not meet the claim requirement for the presence of GAPDH.

Then, the Examiner states that 'Valverde teaches that complementation of the mutant endogenous GAPDH from three clones harbouring recombinant GAPDHs, the unicellular cyanobacterium Synechocystis, the filamentous cyanobacterium Anabaena and E. coli, are able to fermentate sugars and grow on gluconeogenic substrates'. This may have been somewhat mistyped and may not quite represent the Examiner's intention. Having regard to Valverde et al, page 156, first column, we read this as:

'Valverde teaches that complementation of the mutant (i.e. disabled) endogenous GAPDH from in three E. coli clones harbouring recombinant GAPDHs, from the unicellular

cyanobacterium Synechocystis, the filamentous cyanobacterium Anabaena and E. coli, are able enables E. coli to fermentate sugars and grow on gluconeogenic substrates'

It should be noted however, that these three clones of E. coli do not have GAPN, so this teaching says nothing regarding the effect of having both GAPDH and GAPN. As pointed out above, in Figure 2B, the anti GAPDHN antibody reacts only with clone W3CG/pFVNP1 and not with any of the three clones containing the GAPDH genes.

Alternatively, if the three clones the Examiner refers to did also have inserted the gene for GAPDHN/GAPN, Figure 2B would teach the skilled artisan that reinsertion of the capability of GAPDH production has stopped GAPN production.

Accordingly, the Examiner's conclusion that 'the plant remains functional and its functionality is completed by expression of other recombinant GAPDHs in the same mutant E. coli' is clearly wrong. If, contrary to our reading, an attempt was made in Valverde et al to express both GAPDH and GAPDHN in E. coli at the same time, Figure 2 B shows that it did not work.

We submit that a skilled reader would not have found it obvious to combine the teachings as proposed and would not have had a reasonable expectation that the combination would achieve the desired end.

Respectfully submitted,

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